FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **03230006AA**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **not yet assigned** 10/049374

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/21848	**11 August 2000**	**13 August 1999**

TITLE OF INVENTION

METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

APPLICANT(S) FOR DO/EO/US

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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
not yet assigned 10/049374	PCT/US00/21848	03230006AA

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
☒ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$100.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	11 - 20 =	0	x $18.00	$0.00	
Independent claims	3 - 3 =	0	x $80.00	$0.00	
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00	
TOTAL OF ABOVE CALCULATIONS =				$230.00	
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$115.00	
SUBTOTAL =				$115.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$0.00	
TOTAL NATIONAL FEE =				$115.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				$0.00	
TOTAL FEES ENCLOSED =				$115.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$115.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-2041** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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February 11, 2002
DATE

9/pRTS

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JC13 Rec'd PCT/PTO 12 FEB 2002
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METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

DESCRIPTION

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is related to a method which uses platelet contractile force (PCF) measurements and/or clot elastic modulus (CEM) as clinical markers to allow rapid assessment of a patient's risk of atherosclerosis or a patient's bleeding risk during surgical procedures.

Description of the Prior Art

The interplay between atherosclerosis and thrombosis is complex. Multiple local and systemic thrombotic risk factors have been shown to play a role in the destabilization of the vulnerable plaque and its clinical sequelae. Aside from local factors such as the degree of plaque erosion or stenosis, well known systemic risk factors include cholesterol, diabetes mellitus, tobacco, cocaine, hypertension, elevated fibrinogen, impaired fibrinolysis, activated platelets and products or by-products of the coagulation cascade.

Platelet activation occurs in the acute coronary syndrome[1]. The acute coronary syndrome is a continuum from unstable angina to non-Q and

Q-wave myocardial infarction depending on the extent and duration of ischemia. Reduction in coronary blood flow occurs due to platelet aggregation, vasoconstriction at the site of coronary artery stenosis and endothelial injury. Endothelial injury may result from plaque ulceration, hemodynamic factors, systemic arterial hypertension, cardiac catherization, balloon angioplasty, etc. [2,3,4,5]. It is critical to recognize the acute coronary syndrome in patients who present to an emergency department with chest pain in order to prevent inappropriate discharge and adverse consequences[6,7].

Sensitive assays of individual components of the coagulation cascade have made laboratory evaluation of a biochemical hypercoagulable state possible. Prospective studies have suggested that elevated levels of factor VII, fibrinogen and other markers are associated with the development of ischemic cardiac events. However, traditional risk factors have not explained the increased cardiovascular risk in certain high risk groups such as diabetics. The contribution of platelet activation in patients presenting with an acute coronary syndrome has been well established. Unfortunately, to this point, tests of platelet function have not reflected changes predictive of a hypercoagulable state.

Platelet aggregometry, nuclear imaging techniques, serum markers such as Troponin I and T, P-selectin and E-selectin, intercellular adhesion molecules (ICAM) are some of the tools currently available and under investigation to identify patients with acute cardiac events. Nuclear imaging with technetium-99m sestamibi requires considerable resource utilization and has limited ability to differentiate between ischemia, ongoing infarction and prior infarction. Technetium-99m sestamibi also does not identify the unstable plaque [8,9,10]. Elevations of troponin in patients who have myocardial infarction excluded predict an increased risk for short and long term adverse cardiac events. Their utility in acute events is limited since

some degree of myocardial necrosis must occur prior to their release [11]. Platelet aggregation may be a useful marker for predicting mortality in coronary events [12]. However, aggregation techniques that have been used to evaluate platelet dysfunction have been limited to a few non-cardiac clinical situations [13]. Measurement of P-selectin [13], ICAM-1 and/or E-selectin [14] as early markers of platelet activation is ill suited to an emergency department setting because the techniques of flow cytometry and ELISA are time consuming, require technical expertise and need substantial dedicated equipment. Newer methods to assess platelet function are needed.

The Hemodyne® Hemostasis Analyzer is an instrument which measures platelet activity (platelet contractile force, PCF) and clot strength (clot elastic modulus, CEM) in physical units of dynes & dynes/cm^2 respectively[15,16]. U.S. Patents on which the Hemodyne® Hemostasis Analyzer is based include U.S. Patent 4,986,964, U.S. Patent 5,205,159, and U.S. Patent 5,293,772, and each of these patents are incorporated by reference in their entirety. Figure 1 schematically illustrates the components of a system similar to that described in these patents, and which is employed in the Hemodyne® Hemostasis Analyzer. A blood sample obtained from a patient is deposited in a sample cup 10 using a syringe 12 or other suitable device. The cup 10 is placed in a base 14, and a head piece 16 is inserted into the cup 10. This causes the blood 18 to distribute itself along the surface of the head piece 16 and up the sides of the cup 10. The force developed during contraction pulls the head piece 16 and base 14 closer together, and this force is measured using sensors connected to either or both the head piece 16 or base 14. To avoid adverse effects of the three dimensional structure on the clot during formation, a force can be periodically applied to the blood 18 during clotting by the head piece 16.

PCF and CEM are potentially useful tools in a variety of clinical situations[17,18,19]. PCF depends on thrombin production, platelet count,

platelet viability and the degree of platelet inhibition[15,20,21]. CEM depends on the fibrinogen concentration, fibrin structure and platelet function[15]. Inhibition of fibrin(ogen) binding to GP IIb/IIIa blockade either by disruption of GP IIb/IIIa or by competitive blockade, inhibits platelet mediated force development and results in clot structures which are substantially less resistant to deformation by outside forces[22].

Currently, a patient is screened for the presence of atherosclerosis by the patient's response to treadmill exercises and/or by cardiac catheterization. Both tests are time consuming and expensive, and catheterization is quite invasive to the patient. It would be helpful to have available a rapid, less invasive test which may identify those at risk for the presence of atherorsclerosis with the associated increased risk of adverse events such as myocardial infarction, peripheral vascular events, and stroke.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method which utilizes rapid recognition and quantification of platelet activation in patients to identify those at risk for adverse vascular events including thrombosis and hemorrhage.

There have been indicatons that PCF maybe elevated in patients with known coronary artery disease (CAD) when compared to normal control[23], and that CEM is elevated in CAD patients and is reduced, but not normalized, by aspirin therapy (Figures 2 and 3 show data illustrating these phenomena). Thus, it is widely acknowledged that platelets play a major role in arterial thrombosis and are thought to be pivotal in the pathogenesis of atherosclerosis. Despite these acknowledged relationships, no laboratory parameter has been demonstrated to be of value in the determination of thrombotic tendency due to platelet activity. Platelet count, the most

commonly measured platelet parameter, does not correlate with thrombotic risk. It is well known that high platelet counts do not imply an increased risk of thrombosis. Other common tests of platelet functions such as the bleeding time and platelet aggregation studies do not correlate with bleeding or thrombotic risk.

This invention provides a methodology where PCF and CEM are used to rapidly assess the risk of a patient for thrombotic events associated with atherosclerosis or with the risk of bleeding associated with deficient platelet function. Prior studies have not demonstrated that these measures could be used effectively as a screen for probable patient risk. In this invention, it is demonstrated that there is a statistically relevant correlation between PCF and/or CEM and thrombotic risk in patients with atherosclerosis. It is also demonstrated that there is a statistically relevant correlation between PCF and/or CEM and a patient's bleeding risk.

In the emergency department, the measurement of PCF and CEM could be used to detect evidence of hyper-platelet function associated with atherosclerosis in patients presenting with chest pain. Since the presence of atherosclerosis is the greatest risk factor for having a myocardial infarction, this piece of clinical evidence could be used to triage patients toward admission to the hospital or discharge to home. If the force is low or normal, the patient is less likely to have atherosclerosis and is therefore at lower risk of having an myocardial infarction. If the force is elevated two standard deviations above normal, the patient is at high risk even if the clinical history is not compelling.

While PCF and CEM will not diagnosis myocardiaol infarction, they do help identify the most important risk factor and therefore aid in the decision to admit and treat. This is a time consuming and expensive process in the emergency department. Despite the expense and effort, patients are sent home from emergency rooms every day in the United States who are

having a myocardial infarction. Some of these patients die of their event. Conversely, millions are spent admitting and monitoring patients who are not having a mycardial infarction.

Virtually all therapies used in the acute treatment of unstable angina and myocardial infarction result in a decline in PCF and CEM. Heparin anticoagulation, blockade of the platelet receptor glycoprotein IIb/IIIa (by Reopro, Integrilin or Aggrastat), and infusion of nitroglycerin all decrease PCF and CEM. Thus these parameters are not only useful in the identification of high risk patients, they can be used to monitor response to therapy

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of the preferred embodiments of the invention with reference to the drawings.

Figure 1 is a schematic diagram of measurement system used to monitor platelet contractile force and clot elastic modulus during clot formation in whole blood. Anticoagulated whole blood is placed in a shallow conical cup and clot formation is initiated by the addition of clotting agent. Prior to clot formation a conical upper plate is lowered onto the upper surface of the sample, trapping the sample between parallel surfaces separated by a known distance. Platelets within the sample attempt to collapse the clot resulting in a downward force on the upper platelet. This downward force is continuously monitored and the elastic modulus of the forming clot is intermittently measured.

Figure 2 is a graph which shows that preoperative platelet contractile force (PCF) is elevated in patients with documented coronary artery disease

(CAD) who are undergoing coronary artery bypass grafting (CABG). The forces are higher in all such patients but are much higher in such patients who are not taking aspirin. Aspirin appears to decrease but does not normalize PCF values.

Figure 3 is a bar graph which shows the effect of aspirin on whole blood clot elastic modulus (CEM) in patients with documented CAD who are undergoing CABG. CEM were measured at the time of maximal clot retraction. Values for patients with CAD taking or not taking aspirin were significantly elevated over those of asymptomatic control volunteers ($p<0.0002$).

Figure 4 is a bar graph which shows PCF is elevated in patients presenting in the emergency department with a complaint of chest pain. Samples for PCF and CEM determinations were obtained from 99 such patients soon after their arrival in the emergency room. The technician performing the assays did so without knowledge of the patient's clinical status. Upon presentation patient PCF values were significantly higher ($p=0.000000449$) than those seen in 50 asymptomatic volunteers.

Figure 5 is a bar graph which shows PCF values increase with the severity of the patient's clinical presentation. While all groups of patients had significantly elevated PCF values, those patients with electrocardiographic evidence of cardiac ischemia (levels II and I) had the highest PCF levels.

Figure 6 is a bar graph which shows CEM is elevated in patients presenting in the emergency department with a complaint of chest pain. Upon presentation patient CEM values were significantly higher ($p=0.0000145$) than those seen in 50 asymptomatic volunteers.

Figure 7 is a bar graph which shows PCF was significantly elevated in chest pain patients who are subsequently documented to have CAD ($p=0.0002$).

Figure 8 is a bar graph which shows CEM was significantly elevated in chest pain patients who are subsequently documented to have CAD (p=0.0041).

Figure 9 is a bar graph which shows PCF is significantly elevated in patients with hypercholesterolemia (p=0.00048).

Figure 10 is a bar graph which shows CEM is significantly elevated in patients with hypercholesterolemia (p=0.0398).

Figure 11 is a bar graph which shows PCF is significantly elevated in patients with diabetes mellitus (p=0.00012).

Figure 12 is a bar graph which shows CEM is significantly elevated in patients with diabetes mellitus (p=0.00037).

Figure 13 is a line graph that shows that in the chest pain study, PCF increased with age in both patient and asymptomatic males. The correlation was statistically significant (p=0.0032).

Figure 14 is a line graph that shows PCF increased with age in a separate study of apparently normal Italian males (p=0.0137).

Figure 15 is a line graph that shows that in the Italian study, PCF did not change with age in females under the age of 60.

Figure 16 is a line graph that shows that PCF increases with platelet count in all populations studied. The slope of the regression line allows calculation of an average force per platelet number for varying populations. Patients with known arteriovascular disease have higher force per platelet values than asymptomatic age matched controls (see table 3).

Figure 17 is a line graph that shows that collagen-induced whole blood platelet aggregation was depressed in patients presenting in the emergency department with a complaint of chest pain. Samples for aggregation were obtained from 99 such patients soon after their arrival in the emergency room. The technician performing the assays did so without knowledge of the patient's clinical status. Upon presentation patient

aggregation values were significantly lower (p=0.000000449) than those seen in 50 asymptomatic volunteers. While all groups of patients had significantly decreased aggregation, aggregation did not vary significantly between the varying risk levels.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The invention contemplates making PCF and/or CEM measurements on whole blood clots obtained from patient samples during clot formation, and then using these measurements as a screen to identify patient's at risk for an adverse vascular outcome. Application of this technique to clinical samples confirmed that clots with low PCF and/or CEM were less hemostatic and placed the patient at risk for bleeding in conditions such as primary fibrinolysis, Glanzmann thrombasthenia and coronary artery bypass procedures. PCF values less than 4 kilodynes after 720 seconds of clotting are abnormally low. Patients with severe thrombasthenia typically have PCF values below 2 kilodynes. CEM is affected by both fibrinogen concentration and platelet function. CEM values less than 14 kilodynes per cm^2 are indicative of deficient clot formation. In addition, application of this technique to clinical samples confirms that elevations of PCF and CEM are associated with arteriovascular disease and increased risk of arterial thrombosis. Specifically, patients with coronary artery disease, hypercholesterolemia, and diabetes mellitus have much higher PCF and CEM values than asymptomatic controls. In addition, patients who present to the emergency department with complaints of chest pain have significantly elevated forces and the degree of elevation increases with increasing clinical risk. PCF increases with age in males. However, while slightly higher in young females than in young males, PCF does not increase

with age in females at least to the point of menopause. Elevated whole blood PCF and CEM values should help identify patients at increased risk of arterial thrombosis due to atherosclerosis and enhanced platelet function. These measurements should prove useful during the triage of chest pain patients in the emergency department as well as the screening of asymptomatic patients with positive family histories or other documented risk factors for atherosclerosis. Since most therapeutic measures used to acutely treat arterial thrombosis reduce PCF and/or CEM, these parameters can also have applications as monitors of clinical response.

Screening of asymptomatic individuals with PCF and CEM measurements could be useful in indentifying patients who might benefit from more invasive and expensive testing. This can be accomplished by testing a small sample of venous blood. If the PCF value is greater than one standard deviation above the mean of normals, greater than 8.5 kilodynes and the patient has a positive family history or other risk factors (diabetes, cigarette smoking, hypercholesterolemia, etc.), then they should undergo additional testing. If the PCF is normal, 6.9 ± 0.7 kilodynes, no additional testing is needed. If the PCF is above 7.6, testing at intervals to assess whether the force is increasing would be appropriate.

Methods

Patient selection

All patients who present to the emergency department (ED) of the Medical College of Virginia/Virginia Commonwealth University (MCV) with symptoms suggestive of cardiac ischemia undergo prompt clinical evaluation which includes a history, physical exam and ECG. 99 patients presenting to ED with chest pain were recruited for this. When appropriate, blood samples, EKG and a brief history were performed by the ED nursing staff prior to the ED physician interview. Blood samples were obtained prior to the initiation of any therapeutic measures. Further management including

early perfusion imaging with technetium-99m was based on the discretion of individual ED physician and a well established chest pain protocol[24]. Table 1 sets for the acute cardiac evaluation and therapy guide under the protocol.

Table 1. Acute Cardiac Evaluation and Therapy Guide

Diagnosis	Treatment
Level 1	
Acute Myocardial Infarction	
ST elevation	t-PA or primary PTCA
Posterior MI	admit CCU
LBBB with strong clinical Suspicion for AMI	
Level 2	
Unstable Angina	
Ischemic ST-depression or	Standard USA protocol
Ischemic T-wave inversion	
Acute onset CHF	
Known CAD with typical symptoms	
Level 3	
Probable Unstable Angina	
Non-ischemic ECG &	Imaging with Technetium–99m
Typical CP>30 minutes	CCU fast-track
Atypical CP > 30 minutes with	If rest nuclear imaging positive
	admit as level 2
Multiple risk factors	If negative –stress cardiolite - ASAP
Level 4	
Possible Unstable Angina	

Nonischemic EKG & Brief typical chest pain or	Rest imaging with Technetium –99m If negative – home with f/u stress in am
Prolonged atypical CP or Cocaine CP	If positive – CCU admit –treat as level 2

Level 5

Noncardiac CP with clear-cut diagnosis	As appropriate

The hospital course for admitted patients was followed for pre-selected endpoints.

Forty-eight controls were also recruited and similar blood samples were obtained. Exclusion criteria for the control population included no current illness, no history of coronary artery disease or cerebrovascular accident, no recent ingestion of nonsteroidal inflammatory agents including aspirin. Samples for the individual tests were run soon after venipuncture. The institutional review board approved the study protocol.

Sample Handling

A single 15-ml blood sample obtained via an aseptic venipuncture prior to any therapeutic measure was placed into evacuated tubes containing 3.8% sodium citrate. Collagen-induced platelet aggregation, measurements of platelet contractile force (PCF) and elastic modulus (EM) were run in duplicates on whole blood.

Platelet aggregation

Platelet aggregation was measured utilizing a Chrono-Log® whole blood lumi-aggregometer. 450µL of citrated whole blood was mixed with 450 µL of saline and placed in an aggregometer cuvette equipped with a

stirring bar. Platelet aggregation was induced by the addition of collagen (3 mg/ml, Chronolog, Havertown, PA) and the change in impedance was monitored for six minutes.

Measurement of Platelet Contractile Force and Clot elastic Modulus/ Clot formation

Human thrombin, greater than 90% alpha, was purchased as a lyophilized powder from Sigma Chemical Co. (St. Louis, MO). The material with a specific gravity of 3000 NIH units/ml was dissolved in water, diluted with 0.10 M NaCl to a final concentration of 225 units/ml, divided into 50 μL lots and frozen at 80°C. Thrombin was free of plasmin and plasminogen. Nanopure water was used in the preparations of all solutions. Clotting was initiated by adding thrombin (1 NIH unit/ml) and calcium chloride (10mM) to 700 μL of whole blood. Force development was measured for 900 seconds.

The Hemodyne® RM-2 hemostasis analyzer (Hemodyne, Inc., Richmond, VA, USA) measures forces generated by platelets within a clot formed between two parallel cone-shaped plates (Figure 1). The temperature of the sample is held constant via thermal control of the bottom cone, which serves as the sample cup. Before gelation, the upper cone is centered above the cup and lowered into the clotting solution. As the clot forms, it attaches to the inner walls of the cup and upper cone. The entire sample volume is contained between the upper and lower surfaces. Once clotting is complete, platelets within the network pull fibrin strands inward transmitting force through the network to the surfaces to which the clot is adherent. Force measurement is accomplished utilizing a displacement transducer coupled to the upper cone. As platelets contract, the transducer produces an electrical output proportional to the amount of force generated.

Platelet contractile force is determined by measuring the amount of displacement (ΔV_2) of the upper cone during the course of the reaction. In order to compensate for the changing rigidity of the fibrin network, a calibrated compressive force ($F_{applied}$) is periodically applied to the sample by means of an electromagnetic solenoid, and the resulting voltage signal (ΔV_1), due to the displacement of the gel, is measured. PCF is then calculated as follows: PCF $= \Delta V_2 \times (F_{applied} / \Delta V_1)$

Clot Elastic Modulus (CEM) is obtained simultaneously with the PCF. The ratio of applied force (stress) to measured displacement (strain) is used to calculate the elastic modulus: CEM = stress/strain. Where stress equals the applied force ($F_{applied}$) divided by the area of application, and strain is the degree of shape change induced by the applied force. In the present case, the strain induced by $F_{applied}$ is measured as the change in gel thickness, which is the same as the change in the gap between the two cones. Strain is recorded as the ratio of the change in gap distance (d_1) to the original gap distance (d_0). Because the gel is a cylinder of radius (r) and length d_0, stress = $F_{applied} / pr^2$, strain $= d_1/d_0$ and CEM = $(F_{applied} / pr^2) / (d_1/d_0)$ The distance moved (d_1) is measured directly by the displacement transducer.

Sestamibi imaging and interpretation

Chest pain patients were injected with ~ 20mCi sestamibi in the emergency department (not more than 6 hours after the last episode of chest pain) as per the chest pain protocol. Perfusion images were evaluated by an experienced nuclear medicine attending physician and all data were made available to the physicians treating the physician. For purposes of this study, images were classified as either positive or negative for acute myocardial infarction (MI) or ischemia. A positive study required a discrete perfusion defect with associated abnormalities in wall motion and thickening. Studies

visually interpreted as normal, equivocal or consistent with cardiomyopathy were considered negative for acute coronary syndromes. Normal studies had normal perfusion and systolic function without regional wall motion or thickening abnormalities. Studies consistent with cardiomyopathy showed reduced systolic function on cinematic replay with either normal perfusion or perfusion defects without accompanying segmental wall motion abnormalities.

Endpoints

Patients who were admitted to the hospital were followed for specific endpoints. The primary endpoints were myocardial infarction, death, or urgent revascularization (coronary artery bypass graft surgery (CABG), or percutaneuous transluminal coronary angioplasty (PTCA) during the initial evaluation or within 5 days of admission.

Definitions

Myocardial infarction was defined as CK-MB mass $\geq$ 8.0 ng/dl with a relative index (CK-MB mass/total CK x 100) $\geq$ 4.0. For patients having both MI and revascularization, only MI was counted as an event. Anginal symptoms were considered typical if they were described as pressure, tightness, squeezing, burning, heaviness, crushing, or indigestion, or were similar to prior symptoms of angina.

Statistical analysis

Results were presented as mean value $\pm$ SD. Comparisons were made using the Student's t-test and chi-square analysis for categoric and continuous variables. A p-value < 0.05 was considered significant. The relative risk was calculated for various variable correlation coefficients.

Results

Baseline Demographics

The baseline demographics in the patients with chest pain and control patients are given in Table 2. The mean age was 52.8 ± 13.9 (23-87) in the chest patients as compared to 37.7 ± 10.1 (19-62) which was statistically significant. A significant difference in race and sex were also present. There were more blacks in the chest pain group and a preponderance of chest pain patients were male when compared to the control population. As expected, the chest pain patients had a greater number of traditional risk factors as compared to the control population.

TABLE 2

Baseline Demographics in Patients with Chest Pain and in Control Patients

	Chest Pain Patients	Control
Number	99	46
Age (years)	52.8±13.9 (23-87)	37.7±10.1 (19-62)
Gender		
Male	54(54.5%)	23(47.9%)
Female	45(45.5%)	25(52.5%)
Race		
Black	71(71.7%)	6(12.5%)
White	28(28.3%)	32(66.7%)
Asian		10(20.8%)
Smoking Status		
Current Smoker	39(39.4%)	3 (6.5%)
H/O Diabetes Mellitus	20(20.2%)	None
H/O Hypertension	14(14.1%)	2 (4.4%)
H/O Hypercholesterolemia	28 (28.3%)	1 (2.2%)
Mean Platelet Count ($x10^3$)	254±76(149-541)	257±53(181-367)

Mean Hemoglobin (g/dl)	13.2±1.8 (9.2-17)	
Mean time to sample run (mts)	149±83.9	91.5±68.3(10-345)

Risk Stratification/ Myocardial Infarction & Revascularization

All chest pain patients versus controls. PCF was significantly elevated (8.60±0.238 Kdynes) in patients presenting with chest pain as compared to controls (6.95±0.214 Kdynes)) (see Figure 4). PCF was highest in patients with more critical chest pain protocol levels (I&II), but was significantly elevated at all levels (see Figure 5). PCF for I & II level patients grouped together versus grouped III & IV levels approached but did not reach statistical significance (p=0.0735). CEM was also significantly elevated for all levels of chest pain patients compared to normals (Figure 6).

Patients with CAD versus controls. Thirty-six of the 99 patients were documented to have coronary artery disease (CAD) by cardiac catheterization or the occurrence of an acute clinical event. PCF was significantly (p=0.0002) elevated in these patients (8.87±0.459 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 7). CEM was significantly (p=0.0041) elevated in these patients (26.81±1.606 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 8).

Patients with Hypercholesterolemia versus controls. Twenty-eight of the 99 patients were documented to have serum cholesterols greater than 220 mg/dL. PCF was significantly (p=0.00048) elevated in these patients (8.68±0.434 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 9). CEM was significantly (p=0.0398) elevated in these patients (25.40±1.742 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 10).

Patients with Diabetes Mellitus versus controls. Twenty-five of the 99 patients were shown to have hemoglobin A1c levels greater than 7.0.

PCF was significantly (p=0.00012) elevated in these patients (9.57±0.591 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 11). CEM was significantly (p=0.00037) elevated in these patients (30.60±2.174 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 12).

Patients with Positive versus Negative Sestamibi. Sixty-four of the 99 patients underwent sestamibi scanning. Fifteen of these sixty-four patients had a positive scan. PCF tended to be higher in patients with positive (9.4±0.8 Kdynes) versus negative (8.2±0.3 Kdynes) although the difference did not reach statistical significance (p=0.08). Similarly, CEM tended to be higher in patients with positive (29.9±2.6 Kdynes/cm^2) versus negative (25.2±1.1 Kdynes/cm^2) although the difference did not reach statistical significance (p=0.07).

Patients with positive clinical endpoints. Seven patients (7.07%) were documented to have suffered a myocardial infarction (MI). An additional five patients (5.05%) underwent revascularization. Total group of MI and revascularization patients was twelve of ninety-nine (12.12%). PCF was significantly (p<0.05) elevated (8.2±0.7 Kdynes) in the positive endpoint group compared to normals (6.9±0.2 Kdynes). Similarly, CEM was significantly (p<0.05) elevated (24.9±1.9 Kdynes/cm^2) in the positive endpoint group compared to normals (21.7±0.6 Kdynes/cm^2).

Effect of Age. PCF increased with age when all males (patients and controls) were considered as one group (Figure 13). This result was confirmed in a smaller Italian study of asymptomatic males (Figure 14). PCF did not increase with age in American or Italian (Figure 15) females. It is to be noted that this result has only been confirmed in females below the age of fifty-five.

Platelet Force Per Platelet (FPP). PCF is dependent upon and increases with increasing platelet concentration (Figure 16). However, the

increased PCF values in chest pain patients were not due to elevated platelet counts (Table 2). Instead, the slope of the force versus platelet concentration plot (Figure 16) was increased in similar plots for CAD and DM patients. Such plots allows the calculation of a new parameter - force per platelet (FPP). Table 3 shows FPP was highly significantly elevated in CAD and DM patients relative to asymptomatic controls.

TABLE 3

Mean Platelet Contractile Force Per Platelet Values for Various Test Groups

Group	PCF/Platelet (Dynes x 10^{-5} / platelet)	p-value
Controls	3.97	
Coronary Artery Disease	5.309	0.000217
Diabetes Mellitus	6.19	0.000743

Platelet Aggregation in Chest Pain Patients versus controls. Collagen induced whole blood platelet aggregation was significantly reduced in patients presenting the emergency department with chest pain (Figure 17). However, the degree of suppression did not correlate with clinical risk levels.

Table 4 contains a complete odds ratio analysis for the chest pain study.

TABLE 4

Odds Ratio Analysis for PCF and EM versus known risk factors for atherosclerosis and coronary artery disease

	PCF		EM	
	OR(CI)	p value	OR(CI)	p value
CAD	2.0(0.5-7.4)	ns	1.3(0.4-4.6)	ns
DM	2.7(1.7-7.3)	0.06	2.7(1.7-7.3)	0.06
Hypercholesterol	1.6(0.6-4.4)	0.31	0.7(0.3-2.2)	0.7

Male	0.6(0.2-1.6)	0.4	1.1(0.4-2.6)	ns
Tobacco	1.1(0.4-2.9)	0.1	0.8(0.3-2.1)	0.8
Age≥60	1.6(0.6-4.1)	0.5	1.4(0.6-3.7)	ns
LVEF≤45%	0.6(0.12-3.3)	0.7	3.9(1.2-12.2)	<0.05
Black Race	2.4(0.7-7.8)	0.2	1.8(0.6-5.5)	0.3

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While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claim.

CLAIMS

We claim:

1. A method for identifying patients at risk for atherosclerosis, comprising the steps:

obtaining a measurement on the blood sample of a patient selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement to a control to identify a patient as being at risk for atherosclerosis, wherein said patient is identified to be at risk when said measurement is elevated relative to said control.

2. The method of claim 1 wherein said measurement is for platelet contractile force and said control is a value ranging from approximately 5.4 to 8.4 kilodynes.

3. The method of claim 1 wherein said measurement is for clot elastic modulus and said control is a value ranging from approximately 18 to 26 kilodynes per cm^2.

4. The method of claim 1 wherein said step of obtaining is performed by measuring clot contraction forces exerted during clot formation.

5. A method for identifying patients having a bleeding risk, comprising the steps:

obtaining a measurement on the blood sample of a patient selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement to a control to identify a patient as being at risk for a bleeding risk, wherein said patient is identified to be at risk when said measurement is reduced relative to said control.

6. The method of claim 5 wherein said measurement is for platelet contractile force and said control is a value ranging from approximately 5.4 to 8.4.

7. The method of claim 5 wherein said measurement is for clot elastic modulus and said control is a value ranging from approximately 18 to 26 kilodynes per cm^2.

8. The method of claim 5 wherein said step of obtaining is performed by measuring clot contraction forces exerted during clot formation.

9. A method of monitoring treatment or therapy of a patient suffering from unstable angina or myocardial infarction, comprising the steps of:

obtaining a baseline measurement on a blood sample taken from said patient selected from the group consisting of platelet contractile force and clot elastic modulus;

providing said patient with treatment or therapy;

obtaining a measurement on said blood sample after said step of providing, said measurement being selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement and said baseline measurement, wherein progress of said treatment or therapy is indicated by a decline in said measurement relative to said baseline measurement.

10. The method of claim 9 wherein said measurement and said baseline measurement both provide platelet contractile force values.

11. The method of claim 9 wherein said measurement and said baseline measurement both provide clot elastic modulus values.

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(54) Title: METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

(57) Abstract: Platelet contractile force and/or clot elastic modulus measurements are used to identify patients at risk for atherosclerosis or for bleeding during surgical procedures or other applications. Measurements which are elevated are indicative of atherosclerosis, and measurements which are reduced are indicative of a bleeding risk.

WO 01/12211 A1

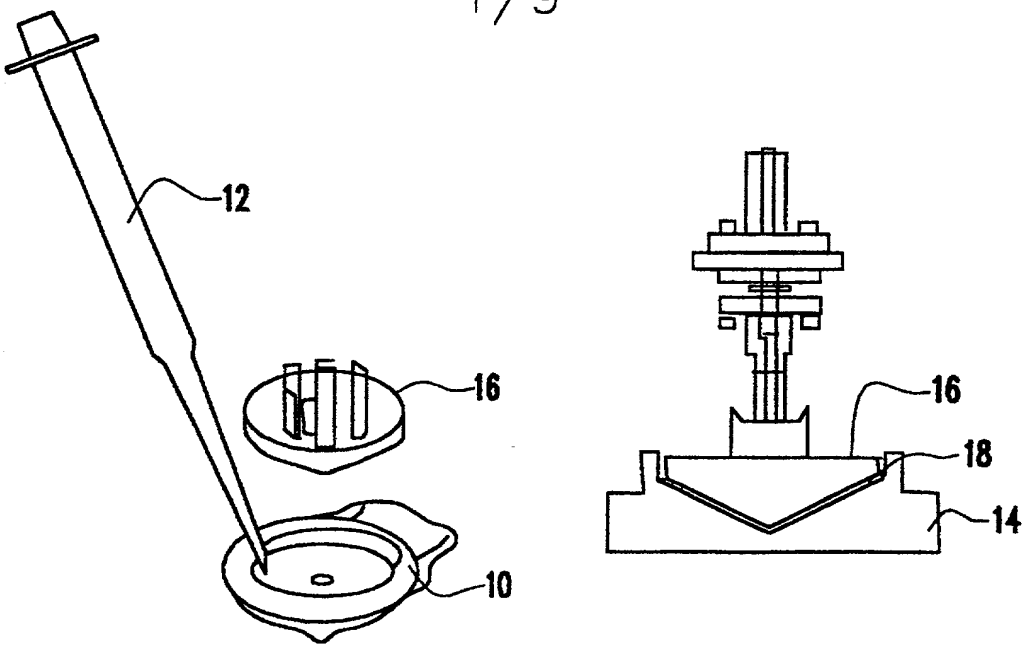

12
16
10
16
18
14

FIG.1

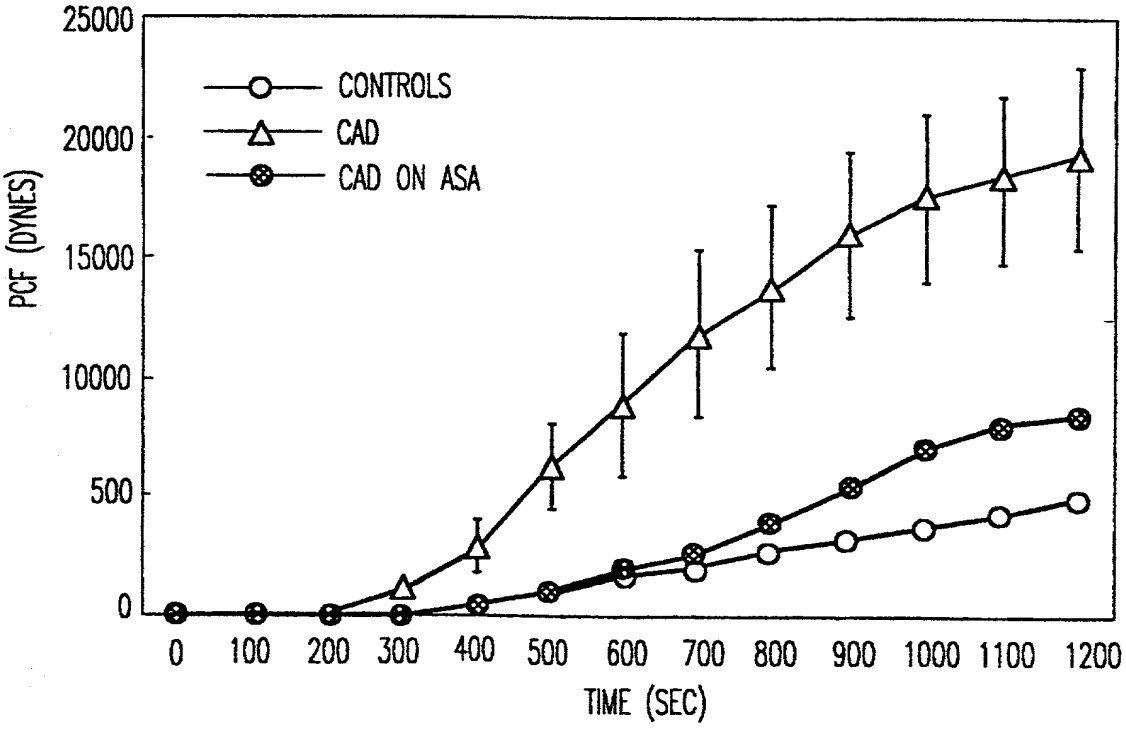

25000
20000
15000
10000
500
0
PCF (DYNES)
CONTROLS
CAD
CAD ON ASA
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
TIME (SEC)

FIG.2

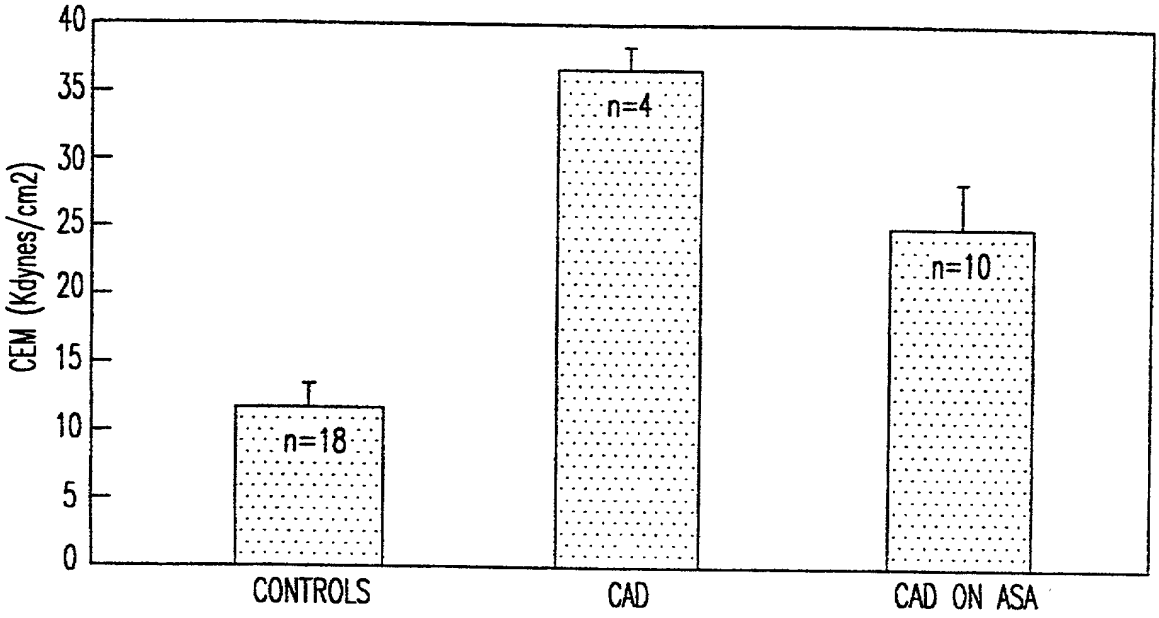
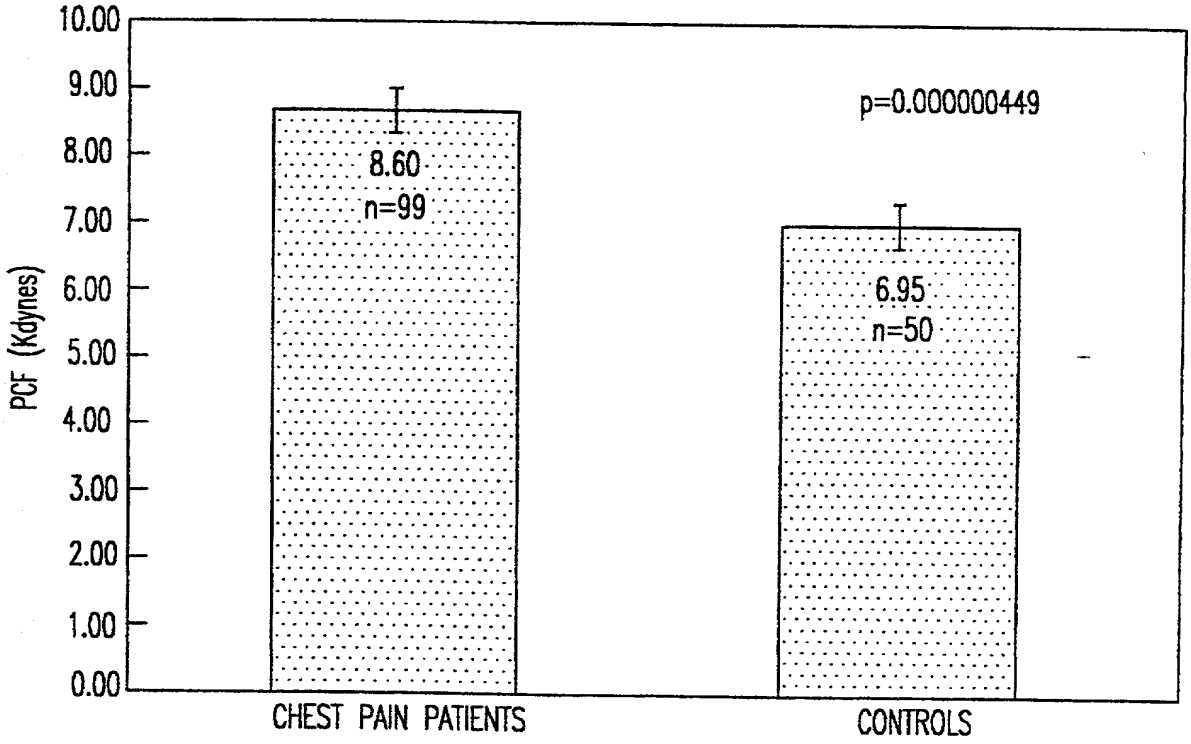

CEM (Kdynes/cm2)
40
35
30
25
20
15
10
5
0
n=18
n=4
n=10
CONTROLS
CAD
CAD ON ASA

FIG.3


PCF (Kdynes)
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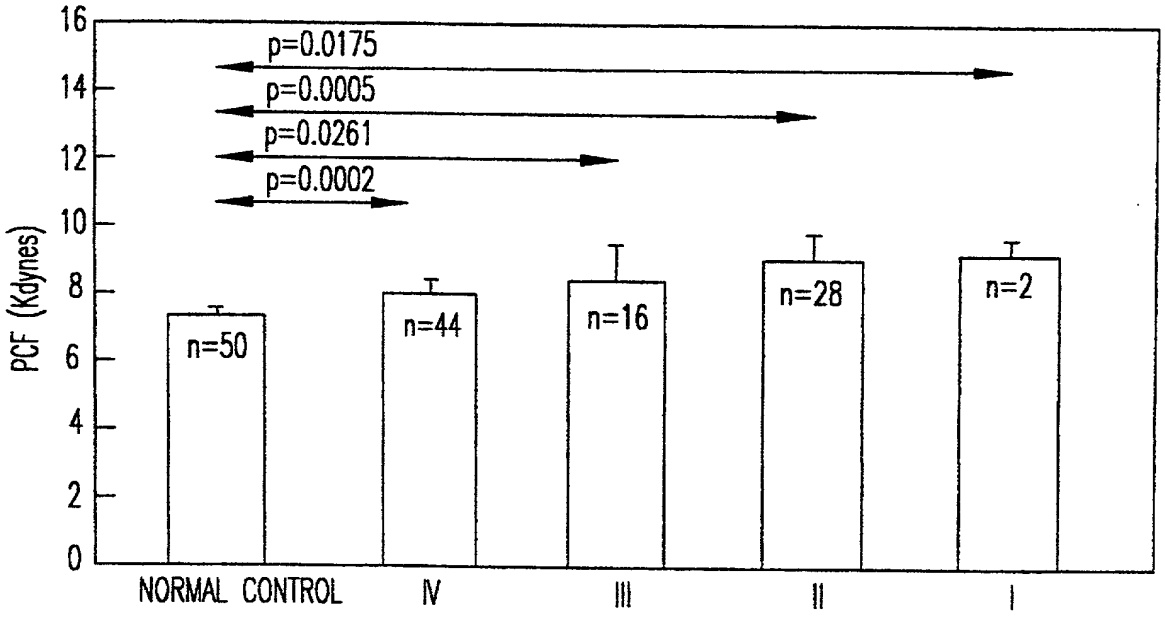
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
p=0.000000449
8.60
n=99
6.95
n=50
CHEST PAIN PATIENTS
CONTROLS

FIG.4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p=0.0005
p=0.0261
p=0.0002
PCF (Kdynes)
16
14
12
10
8
6
4
2
0
n=50
n=44
n=16
n=28
n=2
NORMAL CONTROL
IV
III
II
I

FIG.5

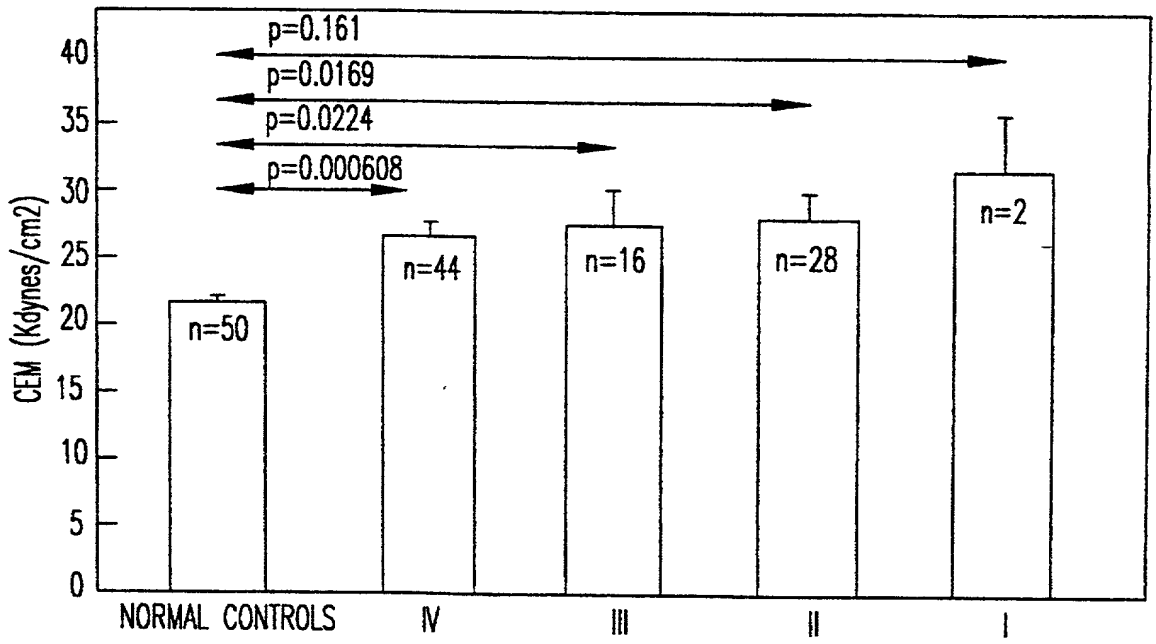

p=0.161
p=0.0169
p=0.0224
p=0.000608
CEM (Kdynes/cm2)
40
35
30
25
20
15
10
5
0
n=50
n=44
n=16
n=28
n=2
NORMAL CONTROLS
IV
III
II
I

FIG.6

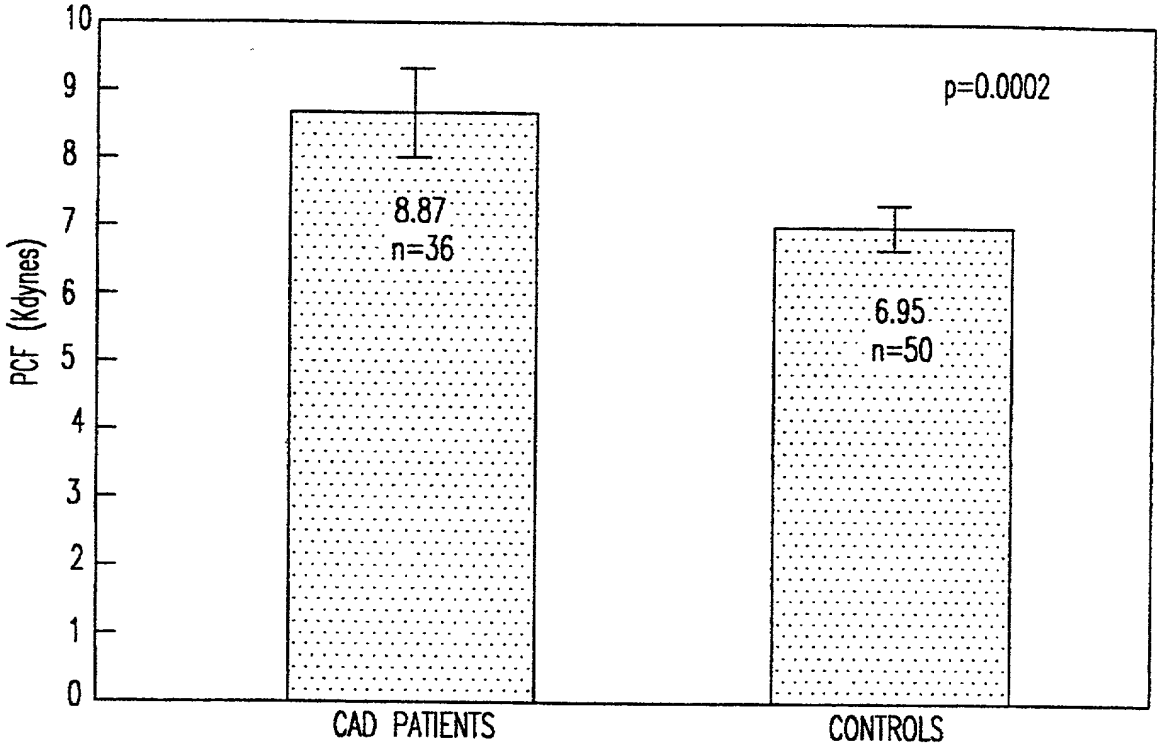

PCF (Kdynes)
10
9
8
7
6
5
4
3
2
1
0
p=0.0002
8.87
n=36
6.95
n=50
CAD PATIENTS
CONTROLS

FIG.7

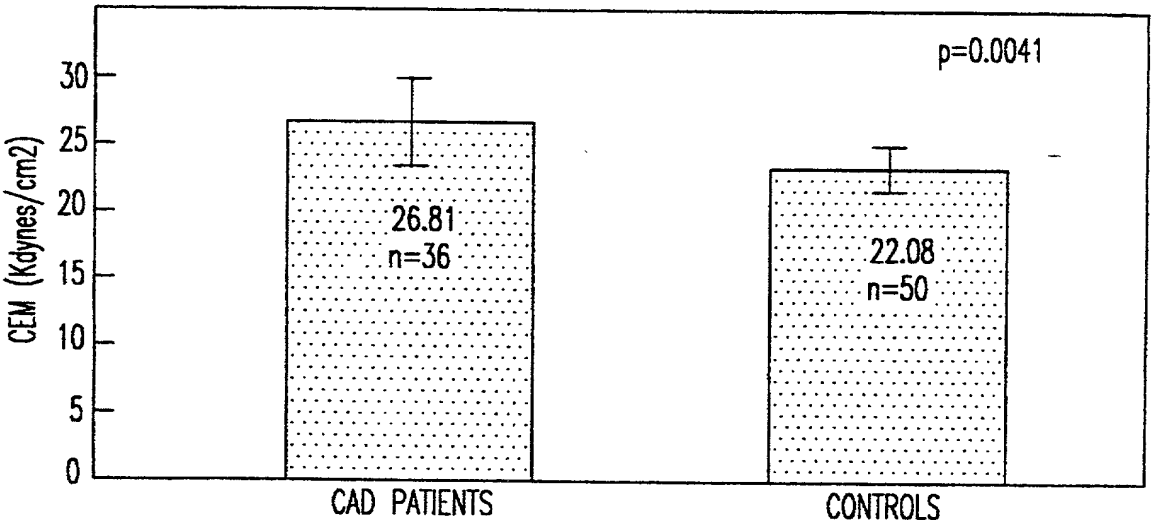
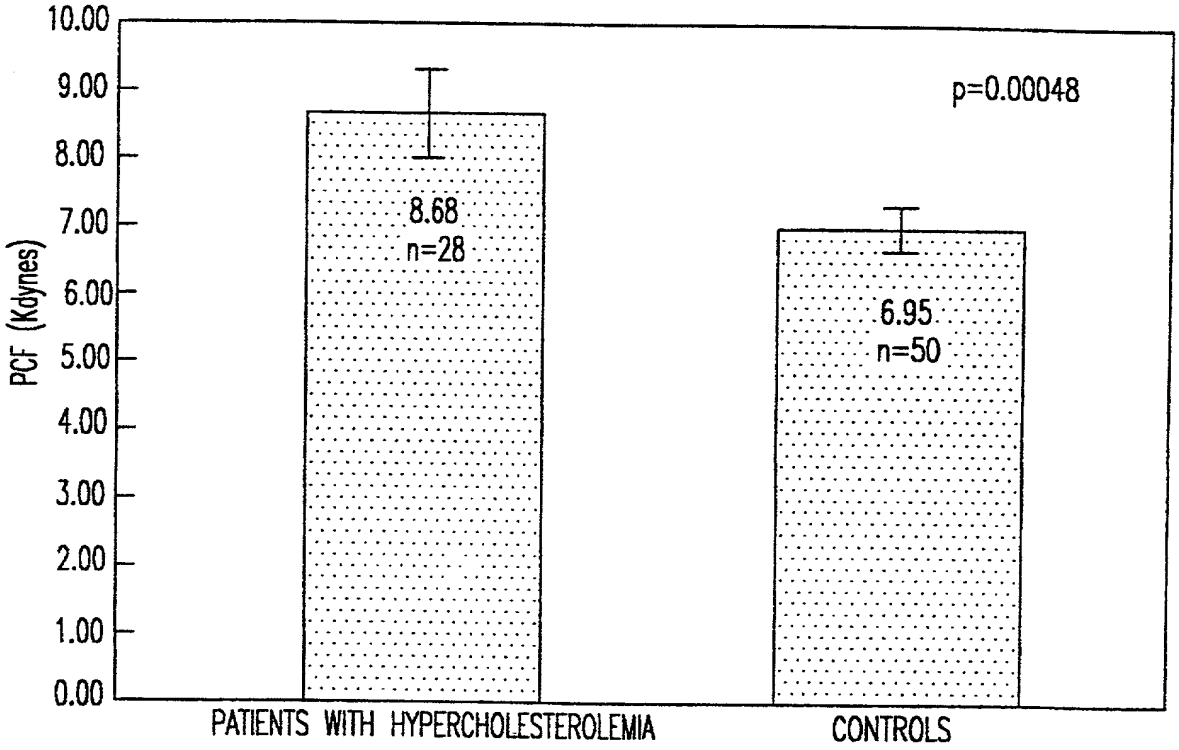

CEM (Kdynes/cm2)
30
25
20
15
10
5
0
p=0.0041
26.81
n=36
22.08
n=50
CAD PATIENTS
CONTROLS

FIG.8


10.00
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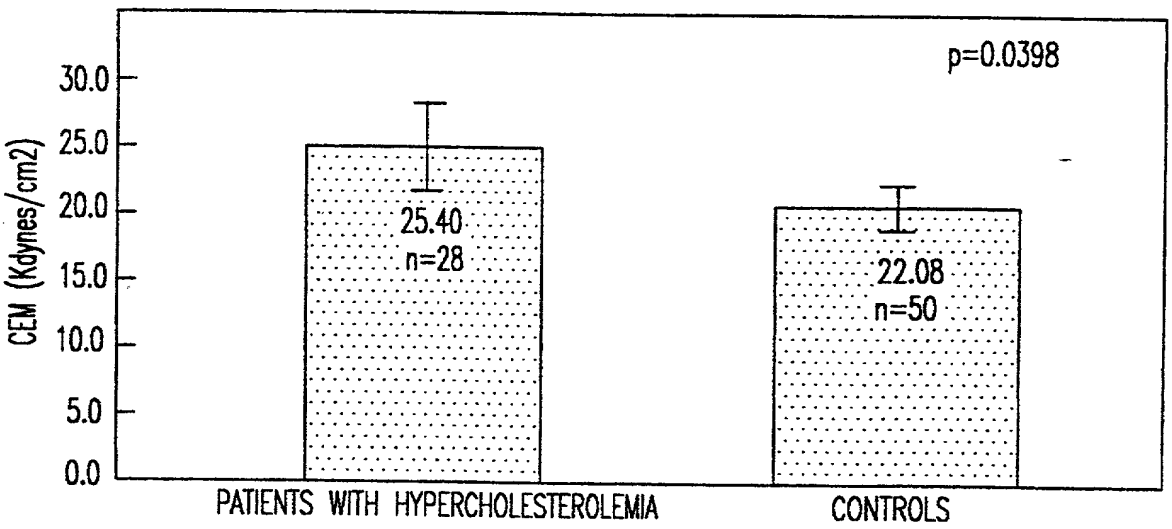
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
PCF (Kdynes)
p=0.00048
8.68
n=28
6.95
n=50
PATIENTS WITH HYPERCHOLESTEROLEMIA
CONTROLS

FIG.9


30.0
25.0
20.0
15.0
10.0
5.0
0.0
CEM (Kdynes/cm2)
p=0.0398
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n=28
22.08
n=50
PATIENTS WITH HYPERCHOLESTEROLEMIA
CONTROLS

FIG.10

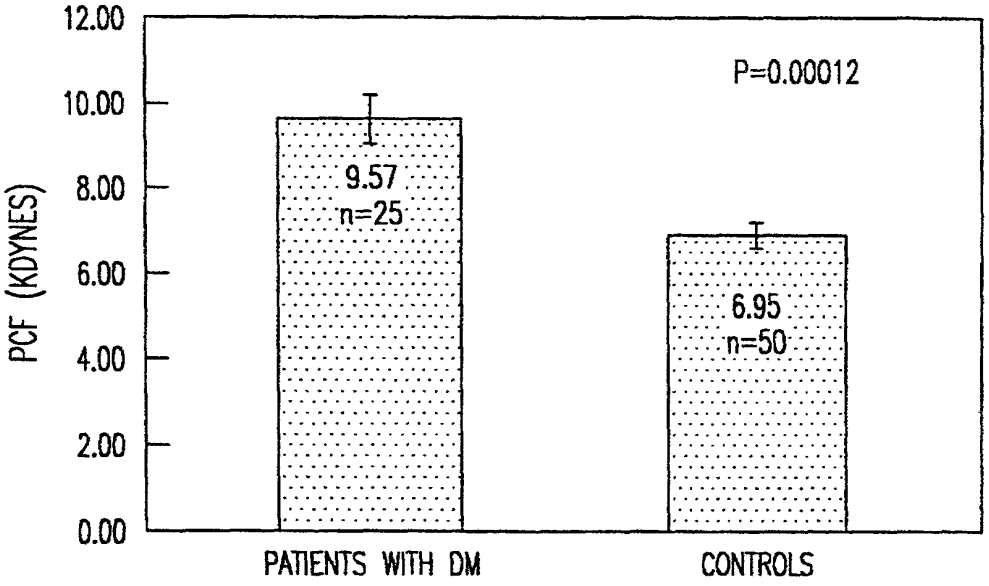
12.00
10.00
8.00
6.00
4.00
2.00
0.00
PCF (KDYNES)
P=0.00012
9.57
n=25
6.95
n=50
PATIENTS WITH DM
CONTROLS

FIG. 11

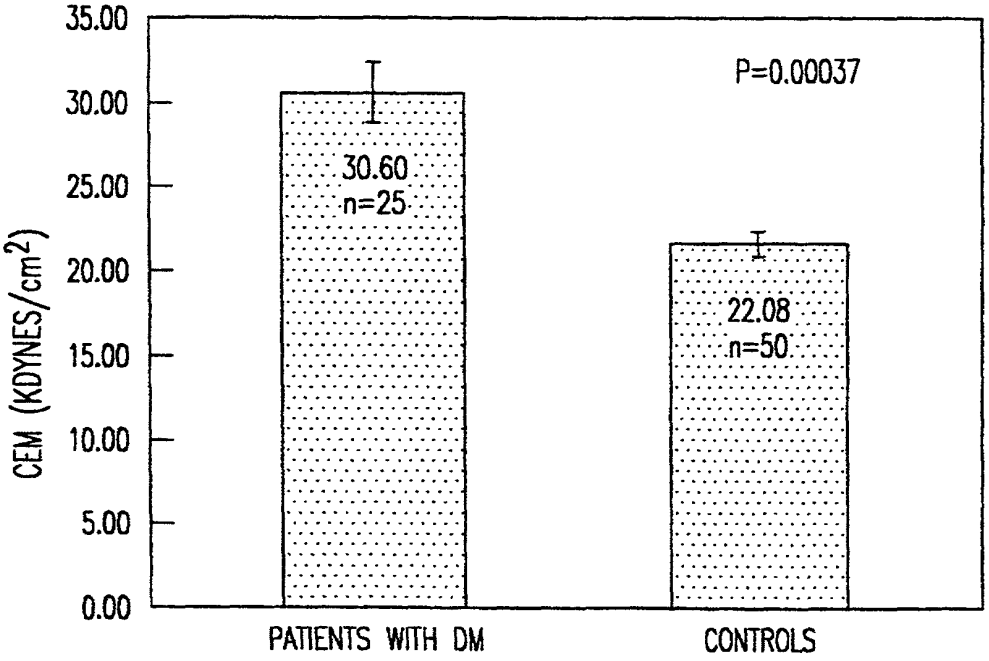
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
CEM (KDYNES/cm2)
P=0.00037
30.60
n=25
22.08
n=50
PATIENTS WITH DM
CONTROLS

FIG. 12

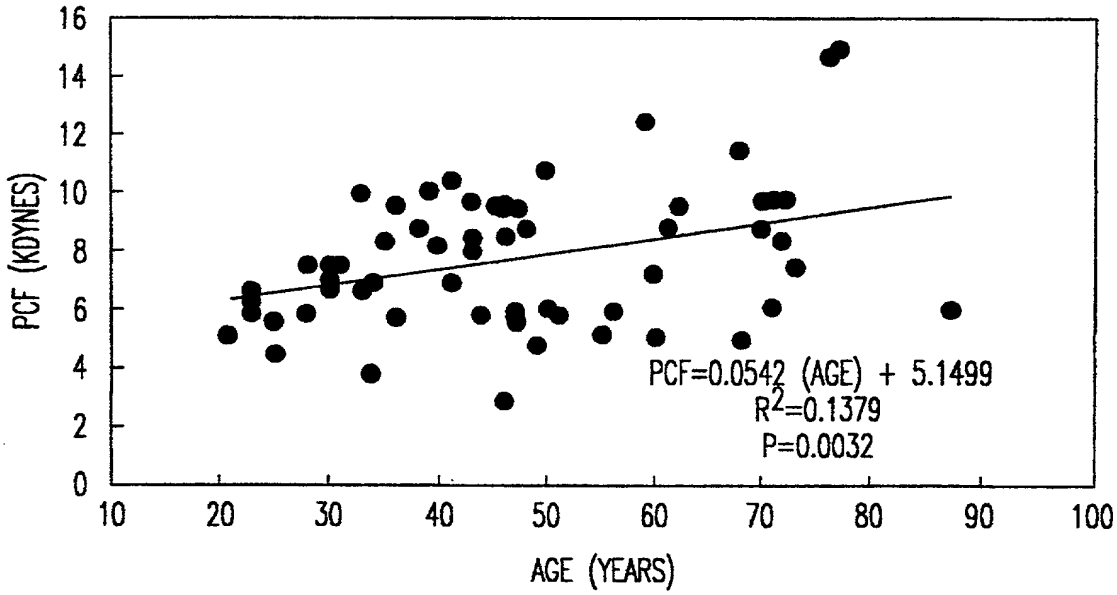
PCF (KDYNES)
AGE (YEARS)
PCF=0.0542 (AGE) + 5.1499
R^2=0.1379
P=0.0032

FIG. 13

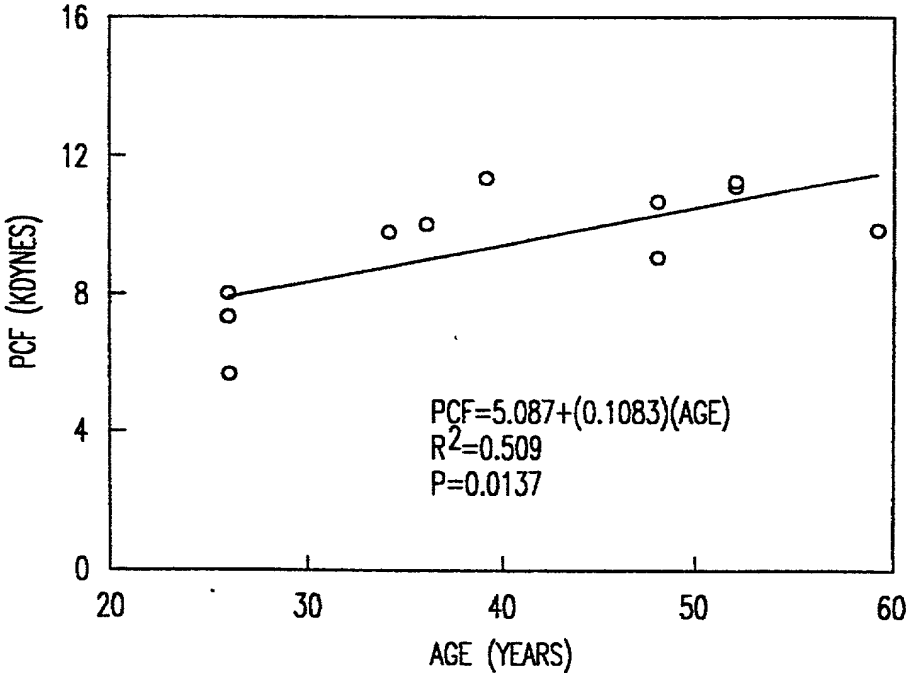
PCF (KDYNES)
AGE (YEARS)
PCF=5.087+(0.1083)(AGE)
R^2=0.509
P=0.0137

FIG. 14

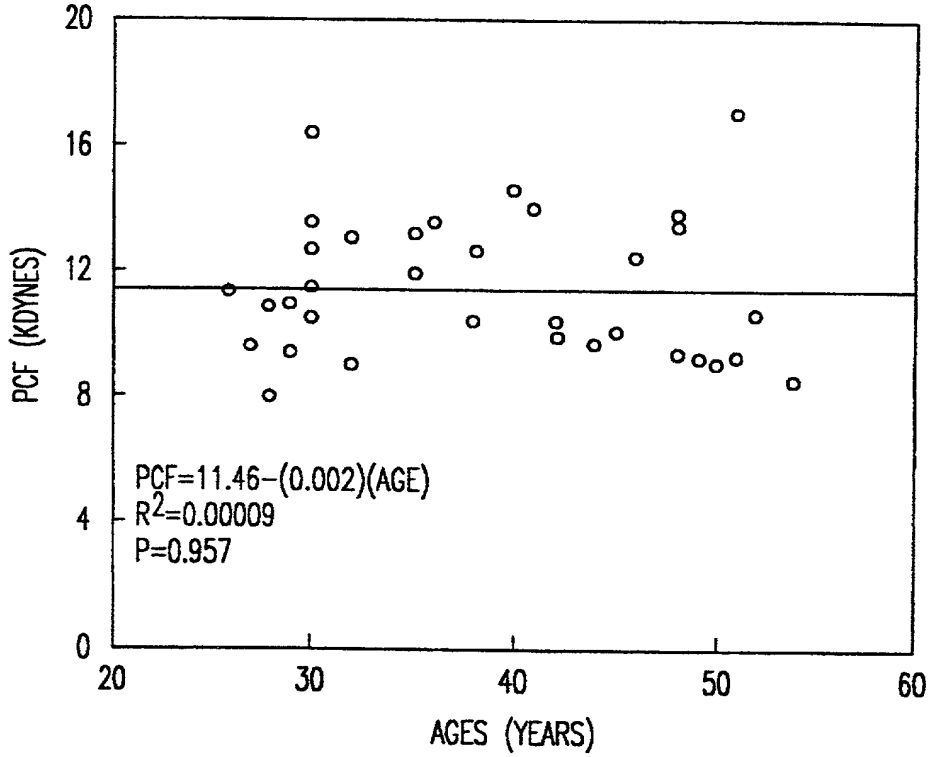
PCF (KDYNES)
AGES (YEARS)
PCF=11.46−(0.002)(AGE)
R^2=0.00009
P=0.957
0
4
8
12
16
20
20
30
40
50
60

FIG. 15

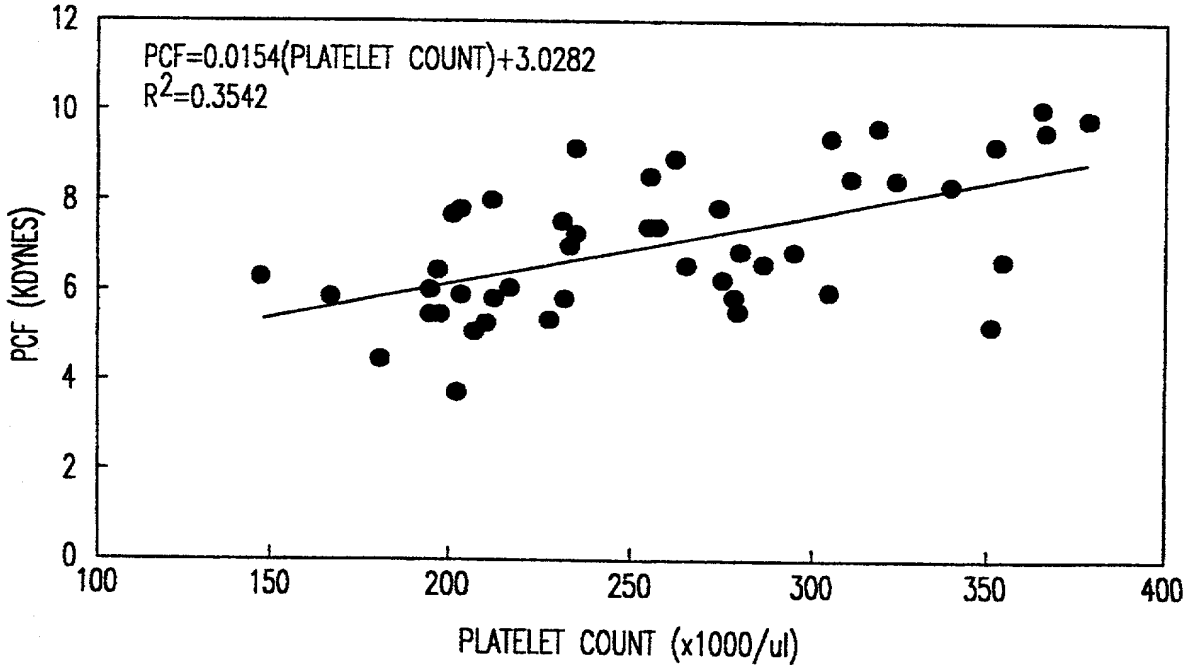
PCF (KDYNES)
PLATELET COUNT (x1000/ul)
PCF=0.0154(PLATELET COUNT)+3.0282
R^2=0.3542
0
2
4
6
8
10
12
100
150
200
250
300
350
400

FIG. 16

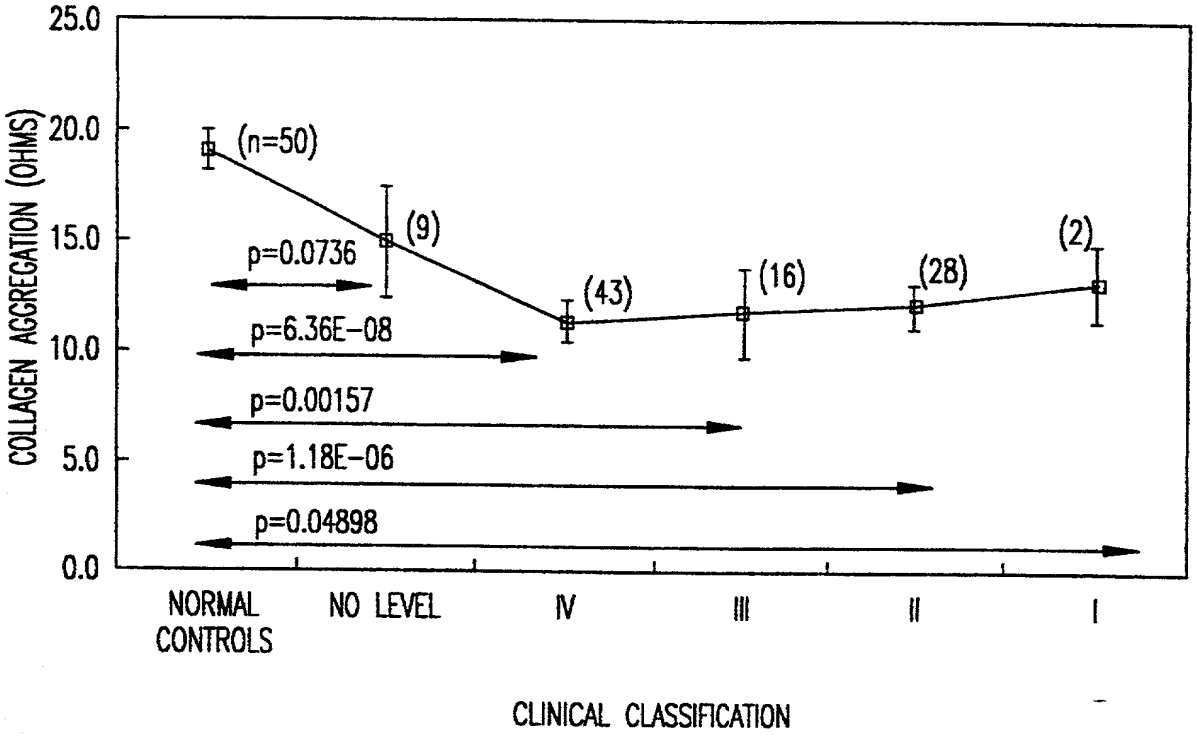
25.0
20.0
15.0
10.0
5.0
0.0
COLLAGEN AGGREGATION (OHMS)
(n=50)
(9)
(43)
(16)
(28)
(2)
p=0.0736
p=6.36E-08
p=0.00157
p=1.18E-06
p=0.04898
NORMAL CONTROLS
NO LEVEL
IV
III
II
I
CLINICAL CLASSIFICATION

FIG. 17

#3

Docket : 03230006AA

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

the specification of which:

(check one)
- ☐ is attached hereto
- ☒ was filed on Feb. 11, 2002
  as Application Serial No. 10/049,374
  and was amended on_____.
  (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56*

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			priority Claimed
PCT/us00/21848	PCT	11/August/2000	X __
(Number)	(Country)	(Day/Month/Year Filed)	yes no

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)
60/148,595	8/13/99	Provisional application

and any continuation applications thereof currently pending.

(4)

Power of Attorney: As a named inventor, I hereby appoint Michael E. Whitham, Reg. No. 32,635, Marshall M. Curtis, Reg. No. 33,138, Clyde R Christofferson, Reg. No. 34,138, and C. Lamont Whitham, Reg. No. 22,424, as attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. All correspondence should be directed to Whitham, Curtis & Christofferson, P.C., 11491 Sunset Hills Road, Suite 340, Reston, Virginia 20190. All telephone calls should be directed to Michael E. Whitham at 703-787-9400.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00
Full Name of Sole Marcus E. Carr, Jr.
Inventor's Signature _____ Date 3/29/02
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2-00
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Inventor's Signature _____ Date 3/29/02
Residence San Jose, California CA
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Post Office Address _____

3-00
Full Name of Joint
or Third Inventor Erika Martin
Inventor's Signature _____ Date 03/29/02
Residence Richmond, Virginia VA
Citizenship United States
Post Office Address _____

Title 37, Code of Federal Regulations, § 1.56:
(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith toward the Patent and Trademark Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned.
(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and (1) it establishes , by itself or in combination with other information, a prima facie case of unpatentability; or (2) it refutes, or is inconsistent with, a position the applicant takes in: (i) opposing an argument of unpatentability relied on by the Office, or (ii) asserting an argument of patentability.